To: Monson, Phil (MPCA)[phil.monson@state.mn.us]; Erickson, Russell[Erickson.Russell@epa.gov]; Hoff, Dale[Hoff.Dale@epa.gov]
Cc: Swain, Ed (MPCA)[edward.swain@state.mn.us]; Engelking, Pat (MPCA)[pat.engelking@state.mn.us]; Kessler, Katrina (MPCA)[katrina.kessler@state.mn.us]; Tomasek, Mark (MPCA)[mark.tomasek@state.mn.us]
From: Mount, Dave
Sent: Thur 5/16/2013 7:54:35 PM
Subject: RE: Wild rice study statistical questions

Ah, got it.

My first concern would be whether two seedlings growing in the same chamber could be considered independent samples for hypothesis testing purposes. My guess is not, though there may be analysis techniques that can be used to deal with this.

My memory is that the previous experiments showed pretty well defined response curves, so I'm not sure whether too few exposure concentrations would be an issue. If the goal were to define point estimates for very low levels of effect (e.g., EC10 or less) then having greater data density in that concentration range would be important, but for EC20 or EC50 values, my memory is that previous experiments seemed to produce data sufficient to define those curves reasonably well.

Again, you should get Russ' input to make sure I'm not off base.

Dave

From: Monson, Phil (MPCA) [mailto:phil.monson@state.mn.us]
Sent: Thursday, May 16, 2013 2:48 PM
To: Mount, Dave; Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: RE: Wild rice study statistical questions

As I understand – at least in general principles, increasing numbers replicates (and I know this is different than number of test organisms) serves to provide for better hypothesis tests while increasing the number of treatment levels in a test serves to better fit a regression-based point estimate. With that said, increasing, if we can, the number of plants (to two plants) in each jar

would/might help to maintain statistical robustness if we choose to run more treatment levels (for needs for regression) and in turn reduce the total number of replicates per treatment to deal with the growth chamber space that we have… Phil

From: Mount, Dave [mailto:Mount.Dave@epa.gov]
Sent: Thursday, May 16, 2013 2:05 PM
To: Monson, Phil (MPCA); Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: RE: Wild rice study statistical questions

Hi Phil—

I may be forgetting something important from those previous discussions, but it's not clear to me why a replication level that provides sufficient power for hypothesis testing would be insufficient for regression. In general, regression approaches tend to be less demanding of replication rather than more. But maybe I'm missing something.

Russ knows way more about it than I, I suspect he'll have something to add.

Dave

From: Monson, Phil (MPCA) [mailto:phil.monson@state.mn.us]
Sent: Thursday, May 16, 2013 12:37 PM
To: Mount, Dave; Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: Wild rice study statistical questions

Hi you guys,

I wanted to give you brief update on our work with developing methods for testing wild rice in the lab.

Sulfate (aerobic) exposures

1. We think we've nailed down pH control (went with a PIPES buffer) and are able to hold it to 6.9 $\pm$ 0.2 pH units.

2. Are in the process of completing another set of range-finder tests using two methods

a. Seeds through germination – 50 seeds per test jar with 3 replicate jars per trt.

b. Emerging seedlings (1-2 day post germination) – 20 tubes per trt with one plant per tube.

Sulfide (anaerobic) exposures

1. Control of pH as described above with still some work needed at higher SO2 (100 micromole)

2. Will perform range finder tests within next month (tent.)

Perhaps the biggest issue will be the best approach for statistical analysis. I think we're in agreement that in order to best describe a dose-response relationship, regression analysis to establish a point estimate will be most favorable. I and Ed are a bit concerned that using only one plant per exposure chamber (tube) may limit the utility of performing a regression. Power test on these tube exposures (I think we shared this information with you at some point) indicated that around 20 tubes should be appropriate replication. That said, I'm toying with the idea to get some larger flasks (double the volume probably around 140 mL) so that we can use two germinating seeds per tube. I'd like your thoughts on this if you have time. In addition, discussion of an appropriate statistical procedure will be a topic at an upcoming (next week) phone conference with the "Technical" Advisory group (sub-group of the larger advisory committee). Perhaps the main point in that will be using regression vs. hypothesis testing as the preferred method and I would like to vet some options to the group for a preferred statistical approach.

Any thoughts you can share would be greatly appreciated.

Thanks!

Phil

Philip Monson

Research Scientist

Minnesota Pollution Control Agency

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